

The following pages of the Karnal Bunt Manual have been updated:

Chapter 3: Regulatory Procedures

Pages 3-4, 3-11, 3-13, 3-14, and 3-16

Appendix D: Laboratory Procedures

Page D-2

Appendix E: Compliance Agreements

Pages E-5, E-21, and E-23

If you are maintaining a print-out of this manual, remember to remove the old pages and insert the corresponding new pages.

3. The Deputy Administrator, through the National Regional Directors, will notify State cooperators of the Karnal bunt detection, actions taken, and actions contemplated. USDA and cooperators will develop and provide a description of the regulated area with support documents to the national program planning staff or its equivalent.
4. APHIS Regulatory Analysis and Development Staff (RADS) or its equivalent will publish emergency regulations under the Plant Protection Act.
5. After a reasonable time to determine the scope of the infection, a proposal to promulgate a quarantine under the Plant Protection Act will be published. The proposal will announce a date for submitting written comments, which shall be approximately 60 days after publication.
6. After receipt of written comments, a final determination specifying the action decided upon will be published in the *Federal Register*. A quarantine will be enacted if eradication has not been achieved under the Plant Protection Act.

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## Certification of Grain

Certify grain at the field or facility level by issuing PPQ Form 540s or local equivalent as approved by the PPQ Project Director.

TABLE 3-1: Grain Certification Decision Table

If the grain is determined to be:	Then:
Bunted Kernel Negative	◆ Use PPQ Form 540 or local equivalent as approved by the PPQ Project Director
Bunted Kernel Positive	◆ Issue PPQ Form 530 (Limited Permit) or local equivalent as approved by the PPQ Project Director for each truck load of grain ◆ Move positive grain only to an approved facility for processing

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## Handling Instructions for Seed Produced Inside the Regulated Area

Spore negative seed produced in the regulated area is only eligible for planting inside the regulated area after treatment. Research breeder germplasm in lots of up to 5,000 lbs can move outside the regulated area after testing negative for spores, **and** after a chlorine bleach treatment consisting of a 1.5 percent aqueous solution of sodium hypochlorite containing 2 mL of Tween 20 per liter, agitated for 10 minutes at room temperature, followed by a 15 minute rinse with clean, running water and then drying, **and** one of the fungicide treatments listed under **Seed Treatment**.

## Decision Charts for Principal Activities

**TABLE 3-3: Grain from KB Regulated Areas**

If grain is from:	And:	And:	Then:
A field sampled for bunted kernels	Positive	For animal feed	Issue a Limited Permit (PPQ 530) for movement to facilities with a Compliance Agreement <sup>1</sup>
		For milling	Issue a Limited Permit for movement to facilities with a Compliance Agreement for mill waste destined for use as animal feed <sup>1</sup>
		Going to a storage facility or to an export terminal facility	Issue a Limited Permit for movement to facilities with a Compliance Agreement <sup>1</sup>
	Negative	For domestic use or going to an export terminal facility <sup>2</sup>	Issue a Certificate to any location for non-planting uses and limited export uses <sup>2</sup>
Storage facilities sampled for bunted kernels <b>or</b> storage facilities located outside the regulated area that are commingled with regulated grain	Positive	For animal feed	Issue a Limited Permit for movement to facilities with a Compliance Agreement <sup>1</sup>
		For milling	Issue a Limited Permit for movement to facilities with a Compliance Agreement for mill waste destined for use as animal feed <sup>1</sup>
		Going to a storage facility or to an export terminal facility	Issue a Limited Permit for movement to facilities with a Compliance Agreement <sup>1</sup>
	Negative	For domestic use	Issue a Limited Permit for movement to any location for any purpose other than planting
		Going to an export terminal facility	1. Issue a Limited Permit for movement to facilities with a Compliance Agreement. 2. Allow movement to countries with no Karnal bunt certification requirements.



- 1 Conveyances for KB positive grain must be leak proof and covered prior to moving outside the regulated area.
- 2 Crops from previously tested positive fields are not eligible for export to countries that require an Additional Declaration (AD) on federal phytosanitary certificates.

**TABLE 3-5: Host Crop Hay/Straw Movement Decision Table**

If moving:	And:	And:	Then:
<b>Within</b> the regulated area	No kernels are present <sup>1</sup>	→	Hay/straw cut prior to the dough stage is unregulated
	Kernels are present (collect a 4-pound sample of grain) <b>or</b> a survey has determined the field's status	The field or kernels test KB negative	Issue a Certificate (PPQ 540 or PPQ 527). The producer must maintain a movement log, if necessary, based on local farming practices.
		The field or kernels test KB positive	1. Send certified notification of positive field status to the owner and producer of the field. 2. Issue a Limited Permit (PPQ 530) for host crop hay/straw fed to livestock or used in areas within the regulated area under conditions of a Compliance Agreement. <sup>2</sup>
<b>Outside</b> the regulated area	No kernels are present <sup>1</sup>	→	Hay cut prior to the dough stage is unregulated
	Kernels are present (collect a 4-pound sample of grain) <b>or</b> a survey has determined the field's status	The field or kernels test KB negative	Issue a Certificate (PPQ 540 or PPQ 527). The producer must maintain a movement log, if necessary, based on local farming practices.
		The field or kernels test KB positive	1. Send certified notification of positive field status to the owner and producer of the field. 2. Call the field manager to explain options. 3. Issue a conveyance Compliance Agreement (CA) to move host crop hay/straw under Limited Permit (PPQ 530) to an approved processing establishment that has a CA.

- 1 Host crop hay cut prior to the dough stage is unregulated and does **not** require an inspection. The hay is eligible for a Certificate (PPQ 540), if requested by the producer, but the certificate is not a requirement or a condition of movement.
- 2 See livestock decision table to determine the requirements of livestock being fed KB positive grain.

**TABLE 3-6: Decision Table for Livestock Grazed on Wheat Grain or Host Crop Hay/  
Straw<sup>1</sup>**

If the wheat:	And:	And in:	And fed on:	Then:
Tested <b>negative</b> for bunted kernels				Allow movement of livestock to any area
Tested <b>positive</b> for bunted kernels or cannot be tested	There has been a 5-day clean-out period of feeding	A feedlot	Bunted kernel negative, treated positive grain, or non-host feed at a feedlot under a Compliance Agreement for manure disposition	Allow movement of livestock to any area (provide PPQ Form 540 if requested)
			Other than grain described in the cell above	Do not allow movement of livestock
		Pasture, rangeland, or a cultivated field within the regulated area	A non-host crop and under a compliance agreement for movement of cattle	Allow movement of livestock to any area with a PPQ Form 540
			A host crop or <b>not</b> under compliance agreement	Do not allow movement of livestock
	There has <b>not</b> been a 5-day clean-out period of feeding			

1 Livestock removed from grazing on host crop prior to the dough stage are not regulated.

TABLE 3-8: Cleaning and Sanitizing Decision Table<sup>1</sup>

If item is:	And:	And:	And:	Then:
Seed cleaning or reconditioning equipment	Working in any regulated area and processed host crop material	_____→	_____→	<ul style="list-style-type: none"> <li>◆ Cleaning and sanitizing required before leaving the regulated area</li> <li>◆ Issue Certificate (PPQ 540) after treatment is observed by program personnel</li> </ul>
	Transiting the regulated area	_____→	_____→	<ul style="list-style-type: none"> <li>◆ Cleaning and sanitizing <b>NOT</b> required; follow best management practices for that state</li> </ul>
Grain storage facilities and conveyance equipment (bins, elevators, bags, legs, augers, railcars, etc.)	Stored or transported positive tested host material (kernels or spores)	Will be used in the production, harvest, transportation, or storage of <b>SEED</b>	_____→	<ul style="list-style-type: none"> <li>◆ Cleaning and sanitizing required</li> <li>◆ Issue Certificate (PPQ 540) after treatment is observed by program personnel</li> </ul>
		Will <b>only</b> be used in the production, harvest, transportation, or storage of <b>GRAIN</b>	_____→	<ul style="list-style-type: none"> <li>◆ Cleaning required; follow best management practices for that state</li> <li>◆ Issue Certificate (PPQ 540) after treatment is observed by program personnel</li> </ul>
	Stored or transported negative tested host material; spore status unknown	Will <b>only</b> be used in the production, harvest, transportation, or storage of <b>GRAIN</b>	_____→	<ul style="list-style-type: none"> <li>◆ Cleaning and sanitizing <b>NOT</b> required; follow best management practices for that state</li> </ul>
		Will be used in the production, harvest, transportation, or storage of <b>SEED</b>	_____→	<ul style="list-style-type: none"> <li>◆ Cleaning and sanitizing required</li> <li>◆ Issue Certificate (PPQ 540) after treatment is observed by program personnel</li> </ul>
Combines and other harvesting equipment (thrashers, headers, grain carts, buggies, etc.)	Transiting the regulated area	_____→	_____→	<ul style="list-style-type: none"> <li>◆ Cleaning and sanitizing <b>NOT</b> required; follow best management practices for that state</li> </ul>
	Working in the regulated area	Known to have harvested positive tested host material (bunted kernels)	Moving within or between regulated areas	<ul style="list-style-type: none"> <li>◆ Cleaning required <b>between</b> regulated areas, optional <b>within</b> regulated area; follow best management practices for that state</li> </ul>
			Leaving the regulated area	<ul style="list-style-type: none"> <li>◆ Cleaning and sanitizing required</li> <li>◆ Issue Certificate (PPQ 540) after treatment is observed by program personnel</li> </ul>
		Known to have harvested negative tested host material	Moving between regulated areas, leaving the regulated area, or crop from next field is intended to be used as seed	<ul style="list-style-type: none"> <li>◆ Cleaning and sanitizing <b>NOT</b> required, but optional; follow best management practices for that state</li> <li>◆ Issue Certificate (PPQ 540) if treatment performed</li> </ul>

<sup>1</sup> For handling equipment within the regulated areas of AZ, CA, NM, and TX.

Grain and seed analysis laboratories must be physically separate and personnel working in grain laboratories (or field personnel) should not enter a seed analysis laboratory without proper decontamination safeguards.

The details of these procedures may be modified to individual laboratory needs; however, basic analysis methods and contamination safeguards should not be deviated from. Some seed analysis laboratories may wish to do the spore wash first and then the analysis of bunted kernels from the remaining dry sample afterward, and that is permissible.

Be very careful to maintain the identity of all grain and seed samples, with careful, accurate labeling.

The techniques for extracting teliospores by selective sieving were developed by Gary Peterson and Morris Bonde<sup>1</sup>, United States Department of Agriculture, Agricultural Research Service (USDA, ARS), Ft. Detrick, MD. Plant Protection and Quarantine (PPQ) Plant Pathologist Ted Boratynski developed the grain inspection machine for bunted kernel analysis. Arizona Department of Agriculture Plant Pathologist Ron Ykema and others at the State Agriculture Laboratory in Phoenix, Arizona assisted in refinement of all the techniques described here and in the preparation of this manual. Joel Floyd, as PPQ Area Identifier in Nogales, AZ, wrote the teliospore and bunted kernel identification sections.

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## Grain Analysis Methods

Laboratories for bunted kernel detection should be in an enclosed area and equipped with the supplies listed below. A process for logging in samples received should be in place. The area designated for grain analysis must be physically separate from the seed (spore) analysis laboratory.

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<sup>1</sup> Peterson, G. L. and M. R. Bonde. 2000. Size-selective sieving for detecting teliospores of *Tilletia indica* in wheat seed samples. Plant Disease 9:999-1007.

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## HAY PRODUCERS

### Compliance Agreement Stipulations

**Before hay may be moved within and/or out of the regulated area it must be inspected by Karnal bunt personnel. The following distinctions will be made:**

- ◆ Hay cut prior to the development of the kernel will be considered no risk for having Karnal bunt.
- ◆ Hay cut with fully developed kernels will be tested for the presence of bunted kernels. Samples can be taken from bales or from standing wheat around the edges of the field from which the hay was cut. The hay will be regulated according to the results of the testing.
- ◆ Hay cut from previously tested fields will be regulated according to the results of that previous test.

**Hay testing NEGATIVE or hay cut PRIOR TO DEVELOPMENT OF KERNEL will be allowed movement under the following conditions:**

Movement of hay **within and out** of the regulated area:

- ◆ Hay must be inspected/tested free of bunted kernels prior to movement from the production area.
- ◆ If hay has already been moved to a storage area inspection/testing will be done at the storage area.
- ◆ Grower will be issued a Certificate for the number of bales and/or weight inspected/tested free of bunted kernels.
- ◆ The number of bales noted on the Certificate for the location (field or storage area) will be allowed movement within or out of the regulated area. The Certificate must accompany any movement.

**Hay testing POSITIVE for bunted kernels will be allowed movement under the following conditions:**

Movement of hay **within** the regulated area:

- ◆ A grower may move his/her hay from the field of production (land owned/leased by grower) to an area owned by the grower for storage.
- ◆ A grower may move his/her hay from the field of production (land owned/leased by grower) for the purpose of feeding to livestock. Livestock fed on hay tested positive for bunted kernels must be fed on Karnal bunt free feed for a minimum of 5 days prior to movement (see restrictions on livestock movement out of the regulated area).
- ◆ A grower must contact Karnal bunt personnel prior to movement of hay. A limited permit will be issued for movement.
- ◆ Hay must be moved in a manner that safeguards the hay and minimizes the potential for spreading Karnal bunt.



### **Instructions for filling out PPQ Form 540 (Certificate)**

Block #1: Enter the date in the following format: (October 12, 2001).

Block #2: Enter the Void Date in the following format: (October 20,2001). Note: Allow a sufficient amount of time for the shipment to arrive directly to the destination to include any possible delays.

Block # 3: Enter your name/company that is selling the commodity.

Block #4: Enter the location from where the grain is being shipped.

Block #5: Enter name and address of the person/company that is purchasing the grain.

Block #6: Enter license plate number of the trailer and the issuing state.

Block #7: Enter Railcar number.

Block #8:

- A. Enter the quantity in pounds.
- B. Enter the Item name that is being issued a certificate.
- C. Enter KB tested negative. Note: In the bottom two lines of block # 8 enter the following statement: "Produced in an area where Karnal bunt is not known to occur. For non-propagative use only."

Block #9: The signatory and designees authorized by the signatory of the Compliance Agreement sign in this block (Shipping Point Signatory).

*Distribution of copies issued by party under compliance:*

- ◆ WHITE COPY (PART 1): Goes with shipment to consignee
- ◆ GREEN COPY (PART 2): Consignor's Copy
- ❖ WHITE COPY (PART 3): Consignor maintains until requested by PPQ

## KARNAL BUNT NEGATIVE GRAIN DEALER/BROKER

### Compliance Agreement Stipulations

FOR REGULATED ARTICLES TESTING **NEGATIVE** FOR KARNAL BUNT

#### 5. I/We Agree To The Following:

##### Certificates/Permits

- ◆ To receive and safeguard PPQ Form 530 (Limited Permits) and PPQ Form 540 (Certificates). These forms are accountable and must be maintained in a secure location. Only employees designated by the signatory of the Compliance Agreement are approved to handle and complete these documents. The signatory of the Compliance Agreement shall provide a list of designees to the USDA/State Department of Agriculture Karnal Bunt Office. Documents shall be completed by following the instructions that are included as attachments to this Compliance Agreement.



Bin-tested negative grain is eligible for a Limited Permit (PPQ Form 530).  
See [Instructions for Filling out PPQ Form 530 \(Limited Permit\)](#).  
Field-tested negative grain is eligible for a Certificate (PPQ Form 540).  
See [Instructions for filling out PPQ Form 540 \(Certificate\)](#).

##### Receiving Grain

- ◆ To handle wheat from the regulated area only if it has been authorized to move under PPQ Form 540 (Certificate) or PPQ Form 530 (Limited Permit).
- ◆ To verify, upon receipt of the shipment, the identity and quantities stated on PPQ Form 540 or PPQ Form 530.

##### Storage of Grain

- ◆ To notify the USDA/State Department of Agriculture Karnal Bunt Project Office 24 hours in advance of turning/moving of stored grain.

##### Shipping Grain

- ◆ To notify the Consignee purchasing the grain that it is strictly prohibited to utilize this grain for the purpose of planting outside the regulated area. This grain can only be utilized for planting inside the regulated area after it has tested negative for karnal bunt spores and has been treated with an approved fungicide.
- ◆ To issue PPQ Form 530 (Limited Permit) or PPQ Form 540 (Certificate) on each conveyance of grain moving to another location. With this permit, grain may be moved to locations within the regulated area or to destinations outside the regulated area. This grain has been certified as free of Karnal bunt (bunted kernel free) and may be moved to any potential market (except for planting) both domestic and/or export. **Note:** many foreign countries requiring an additional declaration on the Phytosanitary Certificate (PPQ Form 577) prohibit wheat grain originating within Karnal bunt regulated areas and/or grain that is commingled with grain from regulated areas from fields that have not been tested. We advise before contracts for the exportation of grain are signed you contact the USDA/State office for the importing country's Karnal bunt export certification requirements.
- ◆ To retain original copies of PPQ Form 530 (Limited Permit) or PPQ Form 540 (Certificate) and make them available at the request of the USDA/State Department of Agriculture Karnal Bunt Program Office, and to maintain supporting records of grain movement associated with the permit or certificate.
- ◆ To obtain all Phytosanitary Certificates, for the purpose of exporting grain, from the USDA/State Department of Agriculture Karnal Bunt Project Office.